EMBRYONIC HISTORY OF THE GERM CELLS IN PASSER DOMESTICUS (L.)

BY

HUBERT W. BLOCKER

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY, IN THE UNIVERSITY OF MICHIGAN

1933

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HUBERT W. BLOCKER

Contribution from the Department of Zoology, University of Michigan.

Five Plates (thirty-two figures).

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INTRODUCTION.

After more than sixty years of careful and painstaking research on the part of numerous investigators, the problem of the origin and early history of the germ cells in vertebrates is still an open question. The many who have attacked the problem have worked with a large number of different species, and have arrived at various conclusions—conclusions which are at times con-

cordant and at times contradictory. This is true especially of the class Aves, a group in which the chick has long served as the classic material for germ cell investigation. It occurred to me that perhaps the study of another type, a species entirely distinct from those hitherto used, might throw additional light on the origin and history of the germ cells in birds. The form chosen is the ubiquitous English sparrow, *Passer domesticus* (L.).

This bird has in the past furnished material for various embryological and histological studies. Etzold (1891), and Loisel (1902) studied the seasonal changes taking place in the testes; Foley (1929), the daily spermatogenic wave occurring in the testes of the adult; Slonaker (1921), the development of the eye; Kummerlöwe (1930 b), the changes taking place in the ovary after hatching, etc. Reese (1905) suggested the use of the English sparrow as general embryological material to replace the later stages of the chick. But as far as I am aware, no work has been done on the early history of the germ cells of this species. As embryological material, the English sparrow offers several advantages as well as disadvantages over the larger forms usually chosen for avian research. Material in abundance is easily obtained in season. The small size of the embryos makes total and complete fixation possible at all stages of incubation, even to the time of hatching. These advantages are offset to some extent by the difficulty encountered in removing the small blastoderm from the yolk in the early stages of incubation, but with the aid of a low-power binocular microscope, this is also readily accomplished.

The problem of this investigation is to trace the history of the germ cells in the English sparrow, *Passer domesticus* (L.), from the time of their earliest recognition in the blastoderm to the hatching stage of the young, and to compare the results obtained with those of other investigators working with other species of birds. I wish here to acknowledge my indebtedness and to express my gratitude and appreciation for the help and encouragement extended by Dr. Peter Okkelberg of the Zoological Department of the University of Michigan, under whose direction the work was carried out; and to my assistant in zoology, Mr. L. F. Kalich, at St. Benedict's College, Atchison, Kansas, for aid in collecting material.

NATURAL HISTORY.

Distribution.

There is, perhaps, no non-migratory bird of the order Passeres that has a wider range of distribution than has *Passer domesticus* (L.). Indigenous to the British Isles, it has, aided by the agencies of man, become distributed over nearly all parts of the earth. Knowlton (1909) gives the distribution

of this species as "throughout Europe generally, northern Africa, and most of Asia, extending as far east as Cochin China and Ceylon. It has also been introduced and become thoroughly naturalized in other parts of the world, notably New Zealand and the United States." Since its introduction at Brooklyn, N.Y., in 1851 or 1852, it has invaded practically every state in the Union, and the greater part of Canada. In most regions of its range, it constitutes the dominant species of the bird population.

Breeding Habits.

The English sparrow is extremely prolific. Normally, from three to four broods are reared during the long breeding season, extending from early April to late July. The number of eggs in a clutch is usually five, less frequently three, four, or six. In the collecting of eggs for this investigation, no clutches of six were found. According to STEELE-ELLIOTT (1900), a clutch of six is most unusual and occurs in only about one per cent of the nests.

The nests of the English sparrow, as CHAPMAN (1928) remarks, are made of any available material in any available place. They are, however, usually loosely constructed of hay and feathers and are placed in various locations in barns and other buildings for added protection. It is only late in the season, that some will take to the building of nests in trees. Nests placed in trees are usually clumsy affairs, containing three or four times the normal amount of nesting material, and are always found in well sheltered locations.

The eggs vary greatly in size and coloration. The normal type may be described as whitish gray thickly marked with dark gray or olive blotches, and approximately two by one and one half centimeters in size. Eggs of the first clutches in the season may be roughly divided into two classes: eggs of the normal type described above, which are usually found in clutches of five, and eggs of a smaller, lighter-colored type, more frequently found in clutches of three or four, rarely five. Whether these smaller eggs are laid by young females has, as far as I am aware, never been determined; but there seems to be some evidence in support of this supposition, since clutches of this type are seldom found later in the season. The difference in color pattern, however, continues throughout the season. RANSOM (1861) from his study of the eggs of the English sparrow, makes the following observation: "The eggs vary in shape and color more than any other bird's eggs that I know of." Notwithstanding this great variation among the eggs of different clutches, eggs found in the same nest are always nearly identical in size, shape, and coloration. It is often possible, after robbing a series of five or six nests and placing the eggs promiscuously into a container, to pick out those which were taken from the different nests.

Incubation does not begin until shortly before the full clutch of eggs has been laid. According to the observations of STEELE-ELLIOTT (1900), when the nest is located in a hole, "the female will roost at the side of the nest but in no way adding to their incubation until (taking for instance the laying of a clutch of five) the evening following the laying of the fourth egg, when incubation starts." In my own experience, it was noted that all eggs taken from the same nest were at approximately the same stage of incubation. I also found that if a nest is robbed repeatedly before the full clutch has been laid, additional eggs will be added; in one case, from a single nest, as many as twenty eggs were obtained which, from their size and markings, were judged to have been laid by the same female. A similar instance is reported by STIEVE (1918) for the European daw, *Colœus monedula,* a species which normally rears but a single brood of five or six per season. He found that if eggs are taken from the nest before the full clutch has been laid, additional eggs will be added to make up the full number before incubation begins. To show that this habit is not peculiar to the daw, but is shared by wild birds generally, he quotes ALTUM (1869) who says: "Eine in hohem Masse bemerkungswerte Tatsache ist es ferner, dass in der Regel die Vögel nicht eher brüten als bis die volle Eierzahl im Neste liegt."

The normal breeding season of the English sparrow is given by DUGMORE (1913) as "almost before winter is gone till late in the autumn." To some extent, the breeding season is influenced by climate and weather conditions. In the literature, we find references to nesting, egg-laying and young sparrows, for almost every month of the year. HADFIELD (1879) reported English sparrows building nests, February 22, 1877, and March 2, 1878. BELL (1843) found a nest with four eggs on the 20th December. GOLDSMITH (1898) encountered four young sparrows in February, 1898, which he thought must have been hatched in January. Other dates given for the finding of fresh eggs by different authors are December 12, and December 15; and the building of nests has been reported for every month of the year. These exceptional cases are mentioned in the literature chiefly on account of their rare occurrence, and as out of harmony with the normal breeding habits of this species. READ (1900), it is true, after reporting the finding of a young, naked sparrow at the end of January, makes the statement that "in large factories and workshops where there is a sufficient warmth, the sparrow nests among the rafters all the year round." Nesting, however, does not always imply egg-laying, but as STEELE-ELLIOT (1900) suggests, may be only for sleeping purposes. The last-named author has also noted that when nests are begun as early as the first week in March, the lining of feathers is not added until several weeks later, when actual egg-lying is begun.

The first eggs obtained for the present investigation were collected on April 15, 1931, although many fruitless attempts at locating nests with eggs

were made earlier in the season. The end of the laying season was not definitely determined. However, late in the summer of 1930, an extended search was made during the first week of September, and neither eggs nor young were found in any of the nests examined. Allowing two weeks for incubation, and two to three weeks more before the young usually leave the nest, it seems logical to conclude that no eggs were laid after the end of July.

That fertilized eggs should be laid as late as October or November, or as early as January or February, is difficult to harmonize with the findings of ETZOLD (1891), LOISEL (1902) and others who have studied the seasonal changes taking place in the male gonads during the course of the year. ETZOLD found that the testes of the English sparrow revert in winter to the immature stage "so dass der Hoden im Winterruhe dieselbe Grösse hat wie der Halbflügger." By actual measurement, he found that the increase in size from January to April is, in extreme cases, 336-fold; in other words, the functional testis weighs over three hundred times as much as the one at rest. He found that during this time the body weight remains fairly constant, so that during the winter resting period, the testes make up only .00062 per cent of the body weight, whereas during the mating season, they make up as much as two per cent. The work of LOISEL has shown, moreover, that the changes are equally great histologically. The author maintains that all the progressive changes in the evolution of spermatogenesis may be studied in material taken at intervals from the resting stage in winter to the stage of functional activity in spring. My own observations on the testes of adult English sparrows, taken at intervals of ten to twenty days, from September 20, 1930 to March 15, 1931, fully agree with those of ETZOLD and LOISEL. Testes of sparrows taken from October to January were of a very nearly constant size, being about the size of an ordinary pin-head. From January on, they increased rapidly in size so that by February 20, they had approached the size of an ordinary pea. Confirmatory evidence, that a seasonal change takes place in the testes of wild birds, has been brought forth by BISSONNETTE (1930), and by BISSONNETTE and CHAPNICK (1930), who studied the normal seasonal changes occurring in the testes of the European starling, *Sturnus vulgaris,* and by WATSON (1919) who made a similar investigation of the changes taking place, during the course of the year, in the testes of the greenfinch, *Ligurinus chloris.* These authors all agree that the testes of wild birds vary in size with the time of the year.

MATERIAL AND METHODS.

Material for this investigation was collected at Atchison, Kansas, during the spring of 1931. Most of the eggs were gathered on the premises of St. Benedict's College. A couple of tall church steeples proved to be a particularly

rich collecting field. Within these airy sanctuaries, the birds had, perhaps, been unmolested for years, as was shown by the great amount of old nesting material in every nook and cranny. The place was visited at intervals of about five days, and all nests, old and new, were examined. Since the English sparrow does not begin incubation until just before the full clutch (usually five) of eggs has been laid, the eggs gathered on these trips were usually fresh. To make sure that only fresh eggs were put into the incubator, when a nest containing five eggs was found, one egg was usually sacrificed to determine the stage of incubation. As soon as gathered, the eggs were taken to the laboratory and placed into an incubator kept at a temperature of 39° C. The stage of development attained by the egg of the English sparrow, after a certain period of incubation, is about the same as that given by LILLIE (1919) for the chick. The early sparrow embryos showed, perhaps, a somewhat more advanced stage of development than chick embryos left in the incubator for an equal length of time, but this was attributed to the shorter warming up period for the smaller egg. Eggs left in the incubator for a sufficient length of time usually hatched on the thirteenth day. Eggs collected from sources other than the one mentioned above, were not incubated, but were opened at once as soon as they reached the laboratory, and the age of the embryos determined by comparing them with those timed by incubation.

Throughout the entire process of fixation, dehydration, clearing and imbedding, extreme care was necessary to obtain perfect, unshrunken embryos. Embryos were removed from the eggs in Ringer's fluid warmed to about forty degrees Centigrade. For the removal of very young embryos, a low-power wide field binocular microscope was employed. A great variety of fixatives were tried, but the best results were obtained with Zenker-formol (Helly's fluid), and with a mixture of equal parts of five per cent trichloracetic acid and a five per cent aqueous solution of corrosive sublimate. For general cellular structures, especially for mitotic stages, Bouin's fluid gave very good results. Fixatives containing osmic acid mere usually capricious; good fixation was, however, occasionally obtained with Bensley's, Schridde's, and Benda's methods. Embryos were fixed entire at all stages of incubation, even up to the time of hatching, but in the case of older embryos, the fixative was also injected into the body cavity to insure rapid fixation of the gonads. In the case of a few embryos at the hatching stage, only part of the rump with the attached Wolffian bodies and gonads was fixed. This seemed to offer no advantage, except for the smaller number of slides and cover glasses required.

Dehydration after all fixatives was carried out according to the drop method as suggested by ALLEN (1916). A current of air, delivering about sixty bubbles per minute, was used to agitate the material during the entire process of dehydration. After washing out the fixative with water, except in

the case of Bouin's fluid, which was washed out with fifty per cent alcohol and brought into the schedule at that stage, the material was placed in a five per cent solution of alcohol; into this was dropped twenty-five per cent alcohol at the rate of five drops per minute, until the embryos were in alcohol of approximately this strength. In the same manner, changes to fifty, seventy, eighty-five and ninety-six per cent alcohols were made. The total length of time for dehydration was usually thirty-six to forty-eight hours, although older embryos were left longer in the different strengths of alcohol before changing to the next higher grade than were the younger stages. All embryos were cleared in oil of Bergamot. They were brought into the clearing oil gradually by leaving them in each of the following mixtures for two to three hours: one fourth oil and three fourth absolute alcohol, one half oil and one half alcohol, three fourths oil and one fourth alcohol, pure Bergamot oil. The latter was changed once before imbedding. In the case of extremely young and delicate embryos, the alcohol was first removed with cinnamaldehyde before subjecting the embryos to the clearing process. When cinnamaldehyde was used, is was followed directly by pure oil of Bergamot. For imbedding, a relatively hard grade of paraffin was used. Material was left in the imbedding oven for three or four hours during which time the paraffin was changed four times.

Sections were cut from three to seven micra in thickness. Best definitions were obtained with those cut four or five micra. Embryos up to twenty-nine somites were sectioned with the entire blastoderm; later stages, up to four days, with only part of the blastodisc, and in the case of still older embryos, only the embryo itself was sectioned. On account of the great amount of space taken up by the sections of embryos older than four and one half or five days, an attempt was made to mount only the region containing the developing gonads. Staining was done exclusively in Heidenhain's iron hematoxylin without counterstain. Sections were mordanted in a four per cent solution of iron alum for five or six hours and left in the stain for twelve to eighteen hours. They were destained in a two per cent solution of iron alum. Several other stains were tried, but on account of inferior results, were quickly abandoned. A total of about seventy-five embryos, ranging in age from the primitive streak to the hatching stage were sectioned and serially mounted.

EMBRYONIC HISTORY OF THE GERM CELLS IN PASSER DOMESTICUS.

Characteristics of the Germ Cells.

Descriptions of the primordial germ cells given by various authors for different species of vertebrates, although differing somewhat in details, all

agree in ascribing to these cells certain general characteristics. For all types of vertebrates, they have been described as large cells, with large, clear nuclei, distinct nuclear and cell membranes, and, according to most authors, as containing an abundance of yolk granules. Various other characteristics have been ascribed to the germ cells by different authors as being specifically diagnostic, such as the Golgi apparatus, the nature of the mitochondria, the presence of plasmosomes, attraction sphere, notched nucleus, specific staining reactions, etc. These diverse characters may be due in part to the species investigated, the technique employed, or the stage described. It is easy to see, therefore, that no single character is sufficient to identify the germ cells in all stages.

The most constant characters of diagnostic value in the germ cells of the English sparrow are their large size with distinct cell outlines, large, clear, round or oval nuclei, eccentrically placed, and with a scanty supply of chromatin usually gathered together into one or two masses with radiating strands; also a large attraction sphere, encircling nearly one half of the nucleus, one, or more often, two distinct centrosomes, surrounded by a lighter area, are always found in favorable sections (figs. 5, 8, 20). Yolk granules, which are emphasized by many authors, are not at all conspicuous in the germ cells of *Passer domesticus*. The absence of visible yolk granules in the germ cells of the English sparrow is probably not a property peculiar to the germ cells of this species, but may be due to the fixatives employed for the early stages when yolk granules are usually most conspicuous. In this connection it is interesting to recall the work on the germ cells of the teleost, *Lophius,* by Dodds (1910). He also failed to find yolk granules conspicuous in the germ cells of this species. He says: "The germ cells of most vertebrates so far studied are up to quite a late stage filled with deeply staining yolk spherules which cause them to stand out prominently among the surrounding cells. In my preparations, they are marked in no such conspicuous manner."

The primordial germ cells are most easily recognized in English sparrow embryos of four to four and one half days of incubation, when all the characters outlined above are present. The attraction sphere, especially, stands out prominently and appears almost as if it were a dense shadow cast by the nucleus. It is not nearly so prominent in germ cells of much earlier stages, and at the stage when the germ cells can first be recognized, it is entirely absent. It is necessary, therefore, when tracing the history of the germ cells, to take into consideration the changes undergone by the germ cells themselves, in order to follow them from their place of origin to their final location in the germ glands.

Previous Work on the Early History of the Germ Cells in Birds.

Morphological Results.

As was pointed out in the introduction to this work, the bulk of the work on the origin and early history of the germ cells in the class Aves, has been done on the chick. It was mainly from his studies on the germ cells of this species, that WALDEYER (1870) formulated his theory of the origin of the germ cells from the "germinal epithelium." He first saw the primordial germ cells in the epithelium of the somatopleure in chick embryos of three and one half days of incubation, and thought that they arose *in situ* by a transformation of epithelial cells. The results of nearly all investigations on germ cells during the succeeding decade are substantially in accord with those of WALDEYER. The germinal epithelium theory for the origin of germ cells was thus quite firmly established in the minds of investigators when NUSSBAUM (1880) announced the theory of early segregation, and later (1901) established the fact that the germ cells in the chick antedate the appearance of the germinal epithelium and, consequently, cannot be derived from it. He first found the primordial germ cells in the splanchnopleure of chick embryos during the second day of incubation.

The existence of these two rival theories, which attempted to account for the origin of the germ cells in birds, greatly stimulated research in this field. A large number of investigations were carried out without settling the question definitely one way or the other. The older germinal epithelium theory of WALDEYER remained firmly intrenched in the minds of most investigators for a number of years after the first announcement of the early segregation theory by NUSSBAUM. As late as 1887, SEMON could say: "Nur über die Entstehung der Ureier aus dem Keimepithel ist man einig." After it had been definitely shown that there is a migration of germ cells to the gonad anlagen from outside sources in the lower vertebrates, renewed attempts were made to discover a path of migration for the germ cells in birds. To recognize the germ cells in their extraregional location, various kinds of technique were resorted to; RUBASCHKIN (1910) and TSCHASKIN (1910) thought that they could distinguish the primordial germ cells from somatic cells by the structure of the mitochondria. They found these structures to be granular in the germ cells and rod-like in the somatic cells. WOODGER (1925) seized upon the Golgi bodies as a means of distinguishing germ cells from somatic cells. Many authors (SWIFT, 1914, GOLDSMITH, 1928, etc.) have found the presence of a large attraction sphere a distinguishing characteristic of the germ cells. An abundance of yolk granules in the primordial germ cells has been reported by most authors. DANTSCHAKOFF (1931 a), while admitting the value of these cytoplasmic structures for the

identification of the germ cells, does not wish to rely too much on any single one, but prefers to consider the general "habitus" of the cell with all its distinguishing characteristics. In summing up the important cytoplasmic structures used by various authors for identifying the primordial germ cells, she says:

"Die Centrosphäre ist ein grosses ausgeprägtes Gebilde, das auf die anderen Bestandteile des Cytoplasmas einen gewaltigen Einfluss ausübt. Der Golgiapparat ist gut ausgeprägt und zeigt bestimmte oben beschriebene Eigentümlichkeiten. Die Mitochondrien sind nicht sehr zahlreich, oft von körniger Beschaffenheit, obgleich, wie es uns scheint, nicht ohne Ausnahme."

But aside from all cytoplasmic structures, she says: "Eine Urkeimzelle kann am Kern allein erkannt werden."

None of the investigators before SWIFT (1914) succeeded in tracing back the germ cells in the chick beyond the second day of incubation. RUBASCHKIN (1907) found primordial germ cells in the epithelium of the splanchnopleure at the cœlomic angle of a twenty-seven somite chick embryo. He observed that they migrated from the place of first discovery during he thirty-five somite stage; he also observed a similar migration of germ cells in a thirty-six somite duck embryo. He does not wish to discuss whether the germ cells take their origin from epithelial cells or from some other sources.

Much support was given to the early segregation theory of germ cells by the work of SWIFT (1914), who was able to show that the primordial germ cells of the chick have an extraembryonic origin, being first found in a crescentic area, anterior and antero-lateral to the embryo at about the two somite stage; that they enter the blood vessels of the vascular area and are carried by the blood stream to the mesentery, where they leave the capillaries and enter the splanchnic mesoderm from which they migrate by amœboid movement to the site of the future gonad. As evidence for the correctness of his views, he gives the following argument: In embryos younger than twenty-one somites, the large cells are in the vessels only. In embryos of twenty-one to twenty-five somites, they are in the vessels and in the tissues. In older embryos, they are found in the tissues of the splanchnic mesoderm alone. Sometimes they are found partly in the vessels and partly in the tissues. He recalls the work of DANTSCHAKOFF (1908) on the blood cells of the chick, and suggests the identity of her "entoderm wandering cells" with the primordial germ cells.

The work of SWIFT was repeated by RICHARDS, HULPIEU and GOLDSMITH (1926), and by GOLDSMITH (1928) with identical results. GOLDSMITH, moreover, carried out his investigations from the earliest embryonic stages to adult birds, and thus was able to establish the identity of the primordial germ cells with the definitive sex cells in the gonads. DANTSCHAKOFF (1931 a)

recently made a careful morphological restudy of the history of the germ cells in the chick. Her results are substantially in agreement with those of SWIFT and GOLDSMITH. She demonstrated the identity of the entoderm wandering cells, first described by her in 1908, with the primordial germ cells, as suggested by SWIFT. To check up on her results, and to prove the correctness of her morphological observations, she also had recourse to the experimental method. The results of this work will be described below.

A unique and novel theory on the locus of origin of the germ cells in the chick has recently been advanced by MATSUMOTO (1932). His work upholds the early segregation of germ cells, but in contrast to all previous work on the germ cells of this species, he finds that the place of earliest recognition of the germ cells in the chick, is not the crescentic germinal area of SWIFT, GOLDSMITH, DANTSCHAKOFF, etc., but the posterior portion of the primitive streak including the primitive plate. He finds them in this position during the stages from a ten to fourteen hours incubation. He suggests, moreover, that the original position of the germ cells in the stages prior to the ten hour incubation stage, is the posterior margins of the germ ring. If MATSUMOTO is really dealing with germ cells, then he has succeeded in distinguishing them some ten hours earlier than any previous worker on the germ cells of this species. There are, however, some considerations which make his early discovery of the germ cells in the posterior region of the blastoderm of the chick appear somewhat doubtful. His illustrations (photomicrographs $\times$ 700) of these early stages do not show primordial germ cells with any degree of clearness. In a recent work of CHEN (1932), on the early embryology of the duck, a careful study was made of the primitive streak region both in whole mounts and in serial sections. CHEN, though not specifically looking for germ cells, sectioned and studied histologically, as many as two hundred and twelve early duck embryos, from the unincubated blastoderm to the one to three somite stage. Although this author made a careful study of the cells of the ectoderm and of the entoderm and the part they take in the formation of the primitive streak, and although he made accurate counts of the different positions of the axes of the mitotic spindles, he makes no mention of any peculiar, large cells of a germ cell nature, which, if present, could hardly have failed to attract his attention. MATSUMOTO's work does not support SWIFT's contention that the entoderm wandering cells of DANTSCHAKOFF are really the primordial germ cells.

It is unfortunate that DANTSCHAKOFF's own later histological and experimental work on the germ cells of the chick, proving the identity of the "Entodermale Wanderzellen" with the primordial germ cells, should not have come to the Japanese author's attention. Since the work in review represents only a preliminary report, and since the author was called away to military duty before he was able to carry out a contemplated experimental project in

support of his findings, we cannot at present pass any final judgment upon his results. They are interesting, but must await further verification or disproof. In my own material of the stages corresponding to those in which MATSUMOTO claims to have found germ cells in the chick, I was unable to find any cells that could possibly be looked upon as germ cells.

Experimental Evidences.

After SWIFT (1914) had located the primordial germ cells in the extra-embryonic region, attempts were made to test the correctness of his observations by the experimental method. The first attempt in this direction was made by REAGAN (1916). He reasoned that if the primordial germ cells are first located in a restricted area outside of the embryo, then by removing this area with its contained primordial germ cells, he should be able to obtain embryos without germ cells. The technique for this operation presented many difficulties. Thousands of embryos were sacrificed, but his labors were finally crowned with partial success. He was able to show that no germ cells are found in the gonad region or mesentery of ninety-four, and one hundred and twenty hour embryos which had undergone extraembryonic castration, in the primitive streak stage, by the removal of the crescentic germinal area. One castrated embryo was raised to the twenty-one day stage but it did not succeed in hatching. A dissection of this embryo showed that the reproductive system had retained the ground plan, but contained no germ cells. Although these attempts at extraembryonic castration were more successful than those later attempted by WILLIER (1926), the results were not entirely satisfactory on account of the exceedingly high mortality; yet they served to lend support to SWIFT's theory of the extraembryonic origin of the germ cells in the chick.

A more thorough and successful piece of work was carried out recently by DANTSCHAKOFF (1931 b) as a sequel to her morphological studies mentioned above. It will be recalled that DANTSCHAKOFF had first found the peculiar large cells, which SWIFT later recognized as the primordial germ cells, in the proamnionic region and in the blood of young chick embryos, and had designated them as "Entodermale Wanderzellen." Following the method of REAGAN, but with a greatly improved technique, she set out to determine the relation of the "Entodermale Wanderzellen" to the primordial germ cells. By destroying the crescentic germinal area, by electric cauterization, wholly or in part, she was able to obtain at will, embryos either entirely without germ cells or with germ cells present in reduced numbers, depending upon the amount of germinal tissue destroyed. No gonad anlagen developed in sterile embryos. In a later work (1931 c), she was also able to show that the primordial germ cells give rise to the definitive sex cells of the adult.

The important work of DANTSCHAKOFF seems to show that there is no

essential difference in the time of germ cell segregation between the lower and the higher vertebrates as FIRKET (1920b) believes, and that the germ cells in warm-blooded vertebrates can be traced to a very early stage of the developing embryo, as EIGENMANN (1891, 1895) claims to have done in a cold-blooded vertebrate, the viviparous teleost, *Micrometrus aggregatus*. The conclusion of DANTSCHAKOFF seems justified when she says: "Somit ist die Geschlechtsnatur der E-W-Zellen bewiesen, und der Keimbahn bei einem warmblütigen Wirbeltier in Form einer ununterbrochenen Linie von ganz primitiven amöboiden Zellen sehr nahe den Blastomeren vorgeschoben."

Early History of the Germ Cells in Passer domesticus.

Locus of Earliest Recognition.

The condition in the English sparrow, to be described below, is in general agreement with the findings in the chick as described by SWIFT (1914), GOLDSMITH (1928), and DANTSCHAKOFF (1931a). In the sparrow, also, the germ cells seem to migrate from an extraembryonic position to the gonad region by way of the blood vessels.

The earliest stage at which the germ cells of the English sparrow can be recognized with any degree of certainty is in an embryo of one or two somites. At this early stage, they are located in a crescentic area cephalad and cephalolaterad to the developing embryo, just at the outer margin of the proamnion (fig. 1). There is as yet no mesoderm in this region, and the germ cells are found in the entoderm and in the space between the entoderm and the ectoderm; none are found in the ectoderm. The outer margin of this crescentic germinal area cannot definitely be established, since at the zone of junction, yolk material is so abundant that cell outlines in the entoderm cannot be distinguished.

The germ cells at this stage (fig. 2) offer no such distinctive marks of recognition as are found in later stages. It is only by following their history through older embryos, that they can definitely be made out as germ cells. Even their large size does not always set them off conspicuously, since many of the somatic cells are also quite large at this early stage. The cell outlines of the germ cells, however, are always quite definite, whereas the cells of the soma take on more or less the form of a syncytium. The distinctive germ cell nucleus with its characteristic chromatin arrangement into one or two masses with radiating strands also helps to identify the germ cells at this early stage. Although certain cells may be recognized as germ cells at the one and two somite stage, it is not possible to decide in every case whether a given cell is or is not a germ cell.

This early embryonic position of the germ cells is retained to about the seven or eight somite stage. With the development of the embryo, more and

more of the germ cells leave the entoderm to take up a position between the entoderm and ectoderm. They appear to be more numerous than in earlier stages. This may be because they are now more easily recognized, especially when they appear isolated in the space between the two germ layers. While the germ cells are very numerous in the space between the entoderm and ectoderm at the seven and eight somite stage, they are now rarely found in the entoderm. No mitotic figures are found in any of the germ cells at this stage.

The germ cells of seven and eight somite embryos (fig. 3) are relatively larger than the somatic cells as compared with earlier stages. This difference in size is to be attributed, not so much to an actual increase in the size of the germ cells, as to a decrease in the size of the somatic cells, due to their repeated divisions. Most of the germ cells have become amœboid and a large attraction sphere is present, although it is not yet so distinct as it is in later stages. The presence of the attraction sphere in the germ cells of seven and eight somite embryos, stages in which the germ cells are still in their original position, is strong evidence that the large cells found in a similar position in the one and two somite embryos are really germ cells. We may conclude, therefore, that the large cells found in the crescentic area, anterior and antero-lateral to the embryo, in *Passer domesticus* of one and two somite stages are the primitive germ cells, and must be homologized with the entoderm wandering cells which Dantschakoff (1908) found in the same region of early chick embryos, and which Swift (1914) thinks are the primordial germ cells. This is supported by the histological and experimental work of Dantschakoff (1931 a, 1931 b). Up to this stage, the mesoderm has not yet reached the germ cell area, but soon the horns of the mesoderm invade the space between the two primary germ layers in the region of the proamnion and after the three germ layers are established in this region, most of the germ cells are found in the mesoderm. The invasion of the mesoderm into the proamnionic region is accomplished, not by a migration *en masse* of the compact mesodermal layer, but peripheral mesodermal cells detach themselves from the main mass and migrate singly between the other two germ layers; the germ cells present in this region thus become completely surrounded by mesoderm cells.

The Entrance of the Germ Cells into the Blood Vessels.

Blood islands appear in the mesoderm almost simultaneously with its encroachment between the entoderm and the ectoderm in the region of the proamnion. These blood islands are at first compact masses of differentiated mesenchyme cells which appear very conspicuous in the looser surrounding tissue. Fluid-filled spaces soon appear in these islands; their cells become

separated from one another and gradually take on the appearance of primitive blood cells. As the spaces expand by the accumulation of fluid, the peripheral cells become flattened against the surrounding tissue and completely inclose the central cells. There is thus established a single-layered endothelial lining for the blood islands and the primitive blood channels formed from them. Some of the germ cells appear to become inclosed passively in the blood islands during their formation. There is also evidence that others enter the blood spaces from without by a process of migration through the endothelial lining. In a ten somite embryo, all steps in the migration from the mesoderm into the vessels may be found (figs. 5, 6, 7). From a study of serial sections, it appears that the germ cells do not enter the blood vessels by active amœboid movement, but that the endothelium is ruptured by mechanical pressure of the germ cells against it, or by pressure from the fluid gathered around the cells. The exact nature of their entrance cannot be determined by the examination of fixed and stained preparations. Figure 5 shows a germ cell lying directly against a primitive blood vessel. The germ cell gives no evidence of amœboid movement, and from cases of this sort, it is not possible to determine by what method the entrance into the blood spaces is effected. A case like that represented in figure 6, however, seems to offer some solution to the problem. A germ cell is here represented as rupturing the endothelial wall of a primitive vessel. It appears that the large germ cell, on account of its turgidity, cannot give way to the pressure exerted on the endothelium by the accumulation of fluid in the vessel and is thus forced through it. Another germ cell, shown among the blood cells in the vessel of figure 7, gives evidence of having entered the vessel in the same way, as part of the broken endothelial wall is still visible. After the germ cell has entered the vessel, the endothelial wall is repaired.

It is difficult to say with certainty, precisely at what stage the migration of the germ cells into the blood vessels is completed. It varies, no doubt, in different embryos, but in embryos of eighteen and twenty-two somites, germ cells can be found only in the blood vessels. In embryos of this stage, they are present in the embryonic as well as in the extraembryonic vessels.

The Vascular Migration of the Germ Cells.

During the early period of their sojourn in the blood vessels, there is no difficulty in distinguishing the large germ cells from the much smaller, embryonic blood cells. But the blood cells enlarge rapidly by growth and imbibition of fluid, and may almost approach the germ cells in size. Some of them may even take on a superficial resemblance to germ cells; but by a careful study of their specific characters, the two types of cells cannot easily be confused. For, besides the general characteristics that are peculiar to the germ

cells at all stages, namely, the attraction sphere and the peculiar arrangement of the chromatin in the nucleus, there is also a decided difference in the staining reactions between the germ cells and the blood cells. The nuclei of the blood cells stain deeply with iron hematoxylin, whereas those of the germ cells remain much paler as in earlier stages. The best criteria, therefore, for distinguishing the germ cells during this part of their history, are the structure of the nucleus and the lighter staining reaction of both nucleus and cytoplasm and the ever-present attraction sphere. Since the germ cells are now distributed throughout the vessels of the embryonic and extraembryonic areas, and since no appreciable increase in their total number has taken place, they are much less frequently met with in sections than when they were concentrated in the crescentic area anterior and antero-lateral to the embryo in earlier stages.

Migration of the Germ Cells from the Blood Vessels.

The germ cells are carried about in the blood vessels to about the twenty-five somite stage, when some of them begin to leave the vessels of the splanchnic region. At this stage, germ cells may be found in the blood vessels of the mesentery and in the tissues along the path of migration to the gonad anlagen as well as in the larger vessels; shortly after this time, they become very numerous in the vessels and mesenchyme of the mesentery and splanchnopleure. Just what causes them to stop in their course in this particular region is a problem that is open to speculation. Swift (1914), Firket (1914), and Reagan (1916) seem to think that in the chick there is an attraction of a chemotactic nature between the germ cells and the so-called germinal epithelium. This may be the case, but in the English sparrow, the attraction is not sufficient to prevent many of the germ cells from passing the gonad region repeatedly, since their exit from the blood vessels may take place at any time between the twenty-five and the thirty-six somite stage. In my material, I have occasionally found germ cells in the omphalomesenteric arteries on their way to the vessels of the blastoderm (fig. 13), and from my observations, I have been led to believe that the lodging of the germ cells in the vessels of the mesentery and splanchnopleure is effected through a mechanical process, determined by the size and shape of the vessels of this region. Dantschakoff (1931 a), who made a study of the blood vessels of this region in the chick, found that there is a reorganization of them at the time when germ cells first become lodged in them. She also found, with rare exceptions, that all the germ cells become lodged in branches from the aorta posterior to the place of origin of the omphalomesenteric arteries, where the circulation is slow and the pressure low. According to her theory, any germ cell that enters one of the omphalomesenteric arteries or one of the larger capillaries which allows the germ cell to pass through unhindered, will again

be carried through the circulation until, on a following circuit, it passes the openings of these vessels and chances to enter one of the smaller capillaries. Of the smaller vessels, many are too small to allow the entrance of the large, turgid germ cells at all. The conditions necessary for arresting the germ cells in their course seem to be a vessel with a lumen large enough to allow entrance to the cells and a caliber small enough more distally, to prevent them from passing through. DANTSCHAKOFF found that the small vessels of the region in question are not equally distributed between the two sides of the body in chick embryos, and suggests that the unequal distribution of germ cells to the two sides of the body may partly find its explanation in this fact. FIRKET (1914), who first called attention to this unequal distribution of the germ cells, offered as a possible explanation, the stronger chemotactic power of the better developed left germinal epithelium. SWIFT (1914) and REAGAN (1916) also seem to favor this view. In my material, I also noted that the germ cells are from two to three times more numerous on the left side of the embryo than they are on the right side (fig. 17). Whether the explanation of this phenomenon offered by DANTSCHAKOFF, or that suggested by FIRKET, SWIFT and REAGAN is correct, I was unable to decide; but the evidence at hand seems to favor the views of DANTSCHAKOFF. I have occasionally found germ cells in and about a vessel of the splanchnopleure which would seem to indicate that the germ cells are held captive by a mechanical obstruction of the capillary. Figure 16, for instance, shows a group of six germ cells in and about a vessel of the mesentery, which I have interpreted as a result of the drifting together of germ cells rather than as a result of their mitotic divisions. It might be argued that if the germ cells are held by mechanical pressure between the walls of the vessels, any other large cells or other objects should be held in the same way. The large blood cells are ordinarily too elastic to be held in this way, but cases like that represented in figure 15 seem to indicate that occassionally, if large enough, they may likewise block the vessel and be kept out of circulation. A similar instance of a large yolk globule, lodged in the lumen of a small vessel in the splanchnopleure of the chick is described and figured by DANTSCHAKOFF (1931 a). That the germ cells do not take a direct path from the aorta to the anlagen of the gonads is evident, not only from their long sojourn in the vessels, but also from their presence in vessels leading from the aorta to the extraembryonic area, e.g. in the omphalomesenteric arteries and their branches (fig. 13).

Occasionally, groups, or nests, of germ cells are found in the mesentery or in the blood vessels of the mesentery, and as no mitoses are found in the germ cells until after their arrival in the gonad anlagen, one may assume that the germ cells found in a nest are not the result of cell divisions, but represent rather a piling up of germ cells behind the first that occluded the vessel (fig. 16).

Migration of the Germ Cells to the Germinal Epithelium.

As soon as the germ cells are lodged in the capillaries of the mesentery and splanchnopleure, they become highly amœboid. This may be the result of a change in viscosity and is a characteristic of the germ cells throughout their period of migration from the blood vessels and the mesentery to the germinal epithelium. It is impossible to determine in all cases, just how the germ cells leave the capillaries. In most cases, the large germ cell fills the blood vessel so completely that scarcely a sign of a vessel is evident when it begins its migration. In other cases, it appears that the germ cells force their way between the endothelial cells into the mesenchyme (figs. 14, 21). From the mesentery, the germ cells migrate dorsad and laterad through the mesenchyme until they reach the site of the future gonad. In the course of their migration, they pass very near to the dorsal aorta, at times giving the appearance that they have come directly from that vessel. It seems, however, highly improbable that this is the case. The germ cells in the larger vessels are always nearly spherical, and no case of a germ cell actually leaving the aorta has been observed. Moreover, the rapidity with which the cells of the blood stream—including the germ cells—must course through these vessels, would offer little chance for active migration through their walls.

As more and more of the germ cells are arrested in the capillaries of the mesentery, the number along the path of migration in the mesenchyme of the mesentery and splanchnopleure is greatly increased. They are very numerous in the splanchnopleure of three-day (34-somite) embryos, that is, before the gonad anlagen have made their appearance. They are strictly localized in the mesentery and splanchnopleure of the posterior region of the embryo. None are found anterior to the orgin of the omphalomesenteric arteries. They are found, therefore, only in sections with a double dorsal aorta, for reasons which have been discussed above. Occasionally, germ cells are found in the epithelium of the splanchnopleure, a fact which was interpreted by the older investigators as an indication that the germ cells are transformed epithelial cells. But the evidence shows that germ cells located in the epithelium do not arise *in situ*, but migrate thence from the deeper layers of the mesenchyme. The germ cells seem to have a special predilection for burying themselves in this tissue. In their migration, they push aside, compress, and in various ways deform the epithelial cells in order to take their place among them. Figure 18 shows two germ cells which are about to take their position among the cells of the germinal epithelium. An epithelial cell has been forced aside by an advancing germ cell. The epithelial cells in contact with the germ cell in figure 19 also give evidence of having been forced out of position by the germ cell. In some cases, there are indications that epithelial cells have been completely thrust out into the cœlomic cavity

by advancing germ cells. As additional evidence in support of the idea that the germ cells found in the epithelium in three and four day embryos do not arise *in situ*, one may call to mind that in slightly younger embryos, germ cells are found in abundance beneath the germinal epithelium, but none are as yet in the epithelium itself. Less than one half of all the germ cells succeed in gaining entrance to the germinal epithelium; many remain in the underlying mesenchyme, where by their presence they aid in bulging out the germinal epithelium to form the anlage of the gonad. The "germinal epithelium", in the sense in which it was originally employed by WALDEYER and his followers, is, therefore, clearly a misnomer; for it not only gives rise to no germ cells, but most of the germ cells do not even come in contact with it. In its present signification, the germinal epithelium should be taken to mean only that part of the cœlomic epithelium found in the region of the developing gonads.

It is thus that we find the disposition of the germ cells in a three to three and one half day embryo of *Passer domesticus*. It was in this position that these large cells were first seen by BORNHAUPT (1867) and recognized by WALDEYER (1870) as germ cells. WALDEYER held, as do all adherents of the germinal epithelium theory, that the germ cells found in the epithelium arose *in situ* by a transformation of epithelial cells. ALLEN (1904), GATENBY (1924), and STIEVE (1927) claim to have seen transitional forms between epithelial cells and primordial germ cells in various classes of vertebrates. From the account given above, it will be seen that the germ cells of the English sparrow do not arise in the germinal epithelium as modified epithelial cells, but have a long history previous to their appearance in the germinal epithelium. They are early segregated cells, which from a definite crescent-shaped area of the blastoderm, anterior and antero-lateral to the embryo in the one to eight somite stage, have arrived in the germinal epithelium after a long period of passive migration through the blood vessels, and by amœboid movement through the mesenchyme of the splanchnopleure, have taken up their position in the epithelium and mesenchyme of the future gonad.

Later Embryonic History of the Germ Cells in Passer domesticus.

Development of the Undifferentiated Gonad.

Almost as soon as the first germ cells have reached the site of the future gonad, a reaction takes place in the epithelium of this region. Its cells become columnar, and in places form a layer of two or three cells in thickness. This thickening is first evident in the region of the splanchnopleure where the omphalomesenteric arteries leave the dorsal aortæ. As development proceeds, the thickening of the splanchnic mesothelium continues farther and farther

caudally. There is at the same time a shifting in the anterior region of this epithelium from the splanchnopleure, through the cœlomic angle, to the somatopleure beneath the Wolffian bodies. Germ cells that had entered the epithelium in the splanchnic region are shifted together with the epithelium to the new position. Germ cells, however, continue to enter the epithelium during its entire passage from the splanchnopleure to the somatopleure and even after it has taken its definite position beneath the Wolffian bodies. This shifting of the germinal epithelium, first evident in the anterior region of the future gonad, is continued posteriorly somewhat later, so that sections of the same embryo show the germinal epithelium in both positions, that is, in the somatopleure anteriorly, and in the splanchnopleure posteriorly (figs. 22 and 23).

The shifting of the germinal epithelium from the splanchnopleure to the somatopleure continues until in four to four and one half day embryos, the entire gonad anlage lies in the somatopleure ventral to the Wolffian body. It extends along the anterior two-thirds of this organ, although its antero-posterior as well as its lateral boundaries are as yet indefinite. The germinal epithelium, which may consist of several layers of columnar cells in the region where it is best developed, namely, immediately beneath the Wolffian bodies, becomes reduced in thickness and merges imperceptably with the peritoneum of the cœlome (fig. 17).

When the gonad anlagen have reached their final position, they increase in size by the arrival of more germ cells beneath the epithelium, and by the mitotic multiplication of the germ cells themselves and of the mesenchyme cells. In embryos of four and one half days incubation, mitotic figures are as yet but seldom seen in germ cells (fig. 26). The mesenchyme cells undergo differentiation, become closely packed together, and take on the appearance of epithelial cells. During the progress of this differentiation, the germ cells retain their identity and may be found in all locations of the gonad (fig. 29). Because of the resemblance of the transformed mesenchyme cells to the cells of their epithelial covering, the boundary between the germinal epithelium and the underlying mesenchyme becomes very indistinct. The epithelial layer as a whole, when seen under low magnification, is stained somewhat darker with iron hematoxylin, and here and there traces of a membrana propria serve, to some extent, to separate the two regions. The gonads at this stage are broadly attached to the Wolffian bodies, and their further development is intimately connected with the development of the mesonephroi themselves.

Sex Differentiation.

Those who have studied the gonads of chick embryos up to the stage of sex differentiation quite uniformly agree that the sex cords are derived

from local thickenings of the germinal epithelium, which grow into the underlying stroma. In the male, these cords give rise to the semeniferous tubules of the testis; in the female, they are said to disappear and to be later replaced by a second proliferation from the germinal epithelium. The cords of the first proliferation are supposed to form the medulla, and the cords of the second proliferation, the cortex of the ovary. It has become customary, therefore, to speak of the first set of cords as the medullary cords, and of the second set, as the cortical cords. The beginning of the formation of the cortical cords, which according to SWIFT (1915) takes place after five and one half days of incubation, makes it possible to recognize the sex of the embryo as female.

In *Passer domesticus,* no such formation of cords from the epithelium seems to take place. The epithelium of the early gonads, as far as can be observed, remains of uniform thickness, consisting of two or three layers of flattened epithelial cells. The nuclei of the dense underlying mesenchyme tissue rearrange themselves in such a way as to give the indifferent gonad the appearance of being made up of indistinct tubules or cords. In the male, these cords become more distinct with the development of the embryo; in the female, they gradually disappear before they have taken on any definite tubular structure. At the time when, in the male, these tubules acquire a distinct outline, there is formed in the female, beneath the epithelium of the left gonad, a dense layer of epithelial-like cells, which is the beginning of the cortex. In the right gonad of the female, this cortex is developed only to a very slight extent, or not at all. These changes, just described, make it possible to distinguish sex histologically only at a relatively late stage in the embryonic development of *Passer domesticus.* No cortex is present in either gonad of the female before about the eighth day of incubation. Throughout the period of sex differentiation, the germ cells are found in all parts of the gonads in both sexes, and they seem to take no definite part in the formation of these organs.

Although sex cannot be distinguished histologically in embryos of *Passer domesticus* before about the eighth day of incubation, it can be recognized macroscopically to some extent in embryos of six or seven days of incubation by the much smaller size of the right gonad in the female. But since the right gonad is usually smaller, even in the male, too much reliance cannot be placed on this criterion alone. When the histological changes described above have taken place, the difference in size between the left and the right gonad in the female has become so pronounced as to leave no doubt about the sex of the embryo, even apart from the histological criteria. The various changes that take place during the process of sex differentiation can best be understood through a histological study of the gonads of older embryos.

The Testis.

In an eleven day male embryo of *Passer domesticus,* the right and the left testes are identical in histological structure. They are not disposed symmetrically with respect to the body axis. Both are shifted somewhat to the right, and the anterior end of the right testis is, moreover, sharply curved to the right, so that similar sections of both testes are seen only in serial sections passing through the posterior region of these organs. They are retroperitoneal in position and still somewhat broadly attached to the Wolffian bodies—the right testis more so than the left. A transverse section of a male embryo of eleven days of incubation, passing through the mid-region of the testes, is given in outline in figure 27 to show the general disposition of the urogenital organs at this stage. Mesonephroi and metanephroi are both present at this stage, and it seems that the mesonephroi are still functional. The tubules of the metanephroi are not yet very distinct and these organs are shown in the figure only in outline. The suprarenal capsules have taken on a glandular structure and are, perhaps, already functioning. The two testes are apparently of equal size, but the right is imbedded somewhat in the nephrogenous tissue of the Wolffian body; the left, however, is so far dextral in position as to have already lost its connection with the corresponding Wolffian body. It is attached to the dorsal wall of the cœlome only by a narrow hilum almost directly beneath the dorsal aorta.

Histologically, the testis is made up of a number of distinct structures. Surrounding the organ on the ventral side is the peritoneal epithelium which is continuous with that of the Wolffian body. Beneath the peritoneum, five or six layers of epithelial cells represent the future albuginea. In the stroma of the testis, a number of tubule-like structures are found, none of which, however, have a lumen. Distinct cell outlines are not visible either in the stroma cells or in the germ cells. The nuclei of the germ cells, however, stand out prominently among the stroma cells by their large size, characteristic chromatin arrangement and lighter staining reaction. The nuclei of the stroma cells of the testis are of two kinds: small, flattened, oval nuclei of the same type as those making up the albuginea; and larger, round nuclei, which make up the walls of the tubules. Among the latter are seen the large germ cell nuclei (fig. 32). That the germ cells are not derived from the cells of the tubular walls, seems evident from the difference in nuclear structure of these two types of cells as well as from the absence of any intermediate stages between them. The tubules, as stated, have no lumen, but the spaces between their walls are filled with a very lightly staining material, which makes these structures stand out prominently even under low magnification (fig. 27). Kummerlöwe (1931) has given a description of these structures, which he also found to be present in the right gonads of the female in young embryos

of *Columba livia, Fringilla cœlebs* and *Passer domesticus.* He says: "Der 'Innenraum' erscheint dann von allerhand Plasmastreifungen, sekretähnlichen Substanzen, usw. angefüllt. Bisweilen zeigt er auch Risse und kleine Spalträume, nirgends aber ein wirkliches Lumen." This is almost an exact description of the conditions found in the eleven-day testis of *Passer domesticus.* The eleven-day embryonic testis is essentially the same histologically as the testis found at the time of hatching on the thirteenth day of incubation.

Some further development of the other organs of the urogenital system takes place during the last two days before hatching, especially the development of the metanephroi and the degeneration of the Wolffian bodies, but a detailed discussion of these changes would lead us too far afield.

The Left Gonad of the Female.

While in the male, there is no essential difference in structure between the right and the left testis, we find that in the female, the difference in the two gonads is sufficiently great to merit separate descriptions. It has been pointed out in the account on sex differentiation that the first indication of a developing ovary is the formation of a cortex beneath the epithelium of the gonad. In an eleven-day embryo of *Passer domesticus,* this cortex has become quite voluminous in the left gonad. It has encroached upon the medulla, which at this time is also conspicuous with the numerous lacunæ and blood sinuses many of which are continuous with those of the Wolffian body. Germ cells are present in both cortex and medulla. In the cortex, they are frequently found directly beneath the epithelial layer. They may be in contact with the cells of this layer, and even partly buried in it, but they are never a part of the epithelial layer, nor in direct contact with the cœlome (fig. 30). Mitotic figures are occasionally present and are sufficient to account for the larger number of germ cells present than during earlier stages. In the stroma cells, mitotic figures are far more frequent. As many as eight contiguous cells with spindles have been observed. The position of the left gonad in an eleven-day female embryo may be described as median; it usually lies directly beneath the dorsal aorta and has a broad connection with the Wolffian body from which it projects lika a large tumor (fig. 28).

The Right Gonad of the Female.

The right gonad of an eleven-day female embryo of *Passer domesticus* is an intermediate structure with characteristics of both an ovary and a testis. It resembles the ovary in the absence of any distinct tubules, and in its broad attachment to the Wolffian body. It has decreased in size in proportion as the left ovary has become more voluminous and has taken a more median

position in the body cavity. At this stage, the right ovary consists of only a narrow strip of gonadal tissue with numerous germ cells (figs. 28, 31). A cortex is usually absent, but beneath the cells of the peritoneal epithelium, several layers of flattened epithelial cells, like those of the albuginea of the testis, give this organ a resemblance to a testis. Germ cells are not found among these flattened cells, nor are there any in contact with the peritoneal covering of the gonad. During the progress of regression of this organ, some of the germ cells have been carried inward toward the Wolffian body, and they may occasionally be found among the tubules of that organ. It would hardly be correct to describe the right gonad of a female *Passer domesticus* as an ovary; it is rather an intermediate structure with resemblances to both an ovary and a testis and, perhaps, with the potentialities of either.

The only difference between the right gonad of an eleven-day female embryo and one at the hatching stage is, perhaps, a further regression of the organ in the later stage, but there is also a great deal of variation in this structure in different embryos. During the entire embryonic period, germ cells are present in the right gonad and, for a given amount of gonad tissue, in numbers as great as those of the left ovary.

SUMMARY OF THE LITERATURE ON THE ORIGIN OF THE GERM CELLS IN VERTEBRATES.

The various conclusions arrived at by different investigators on the origin of the germ cells in birds is by no means peculiar to this class. Early segregation and somatic origin of germ cells have been claimed for representatives of all vertebrate classes. In general, early segregation has been more commonly claimed for the lower members of the group than for its higher representatives. It was this fact that lead FIRKET (1920 b) to suggest that the so-called primordial germ cells in birds and mammals are of only phylogenetic importance. It is interesting to note that when the same investigator worked with species belonging to different classes of vertebrates, similar conclusions are arrived at for all. RUBASCHKIN claims early segregation of germ cells for the chick (1912), cat, rabbit (1908), mole, porpoise (1909) and Guinea pig (1912). Similarly, VON BERENBERG-GOSSLER holds to a somatic origin of germ cells for Lacerta (1914) and for the chick (1912). DUSTIN maintains a somatic origin of germ cells for Triton, Rana, and Bufo (1907) and Chrysemys (1910). ALLEN, however, upholds the theory of early segregation in Amia, Lepisosteus (1909), Rana (1907) and Chrysemys (1906), but maintains a peritoneal origin for the germ cells of the pig and the rabbit (1904). It must not be overlooked, however, that in his report on the germ cells of Chrysemys, a work which was carried out subsequently to his work on mammals, he says that "the present work has shown with suf-

ficient clearness certain possible sources of error and misinterpretations" in his former investigations, so as to cast some doubt upon the conclusions arrived at, especially with regard to the apparent transition forms connecting the peritoneal cells with the germ cells. In this later work on Chrysemys, he found that the sex cells lie among the peritoneal cells but are not derived from them.

It would obviously be impossible in this limited space to give proper credit to all the important works that have appeared in this vast field. An excellent summary of the literature is given by Okkelberg (1921), and more recently, McCosh (1930) has brought it up to comparatively recent times by the addition of some thirty articles that have been appeared since 1921.

Several authors have attempted a classification of the current theories on germ cell origin in vertebrates. Hegner (1914) finds it convenient to employ a three-fold classification: the germinal epithelium theory, the gonotome theory, and the theory of early segregation. McCosh (1930) makes use of a somewhat similar classification. She mentions the germinal epithelium theory, the early segregation theory and a "middle ground". Perhaps the most successful attempt at a complete classification is that of Heys (1931). Her system of classification embraces four groups, sufficiently distinct from each other to merit individual attention. Group I is composed of those who deny the early segregation of the germ cells and believe that germ cell formation is a matter of differentiation of somatic cells. In group II, she places those who admit an early segregation of germ cells, but hold that such cells are not definitive, and that they are later replaced by proliferations of new cells from the germinal epithelium. Her group III is made up of those who hold that some of the primordial germ cells persist as definitive germ cells, but that their numbers are increased periodically by a proliferation from the epithelium. Finally, in group IV, she places only those who adhere strictly to the "Keimbahn" of Weismann, and maintain that the germ cells are set aside at an early stage of embryonic development and are the sole source of the definitive sex elements.

Matsumoto (1932) holds that for birds, the various theories on germ cell origin fall naturally into two main divisions: the germinal epithelium theory, and the theory of early segregation. Those who adhere to the latter theory, he subdivides into those who believe that the primordial germ cells are the definitive sex cells, and those who claim that the primordial germ cells degenerate and are replaced later by definitive elements from the germinal epithelium. I would like to extend this dichotomous system of classification of Matsumoto for birds, to the whole vertebrate group, without, however, subscribing to his subdivision of the early segregation group. It seems to me that the main point at issue is whether or not there is a germinal continuity from one generation to another—whether there exists in verte-

	Author and year	Species	Remarks
		CYCLOSTOMES	
Early segregation	Beard (1902)	Petromyzon planeri	The number of early segregated germ cells is 2^5—1.
	Okkelberg (1921)	Entosphenus wilderi	Definitive germ cells take their origin only from early segregated cells.
Somatic origin	Butcher (1929)	Petromyzon marinus unicolor	The definitive germ cells have a dual origin: a) from early segregated cells; b) from cœlomic epithelial cells.
		ELASMOBRANCHS	
Early segregation	Beard (1900) (1902)	Raja batis Pristiurus	Germ cells are early segmentation cells.
	Woods (1902)	Squalus acanthias	The primordial germ cells are first seen in the entoderm.
Somatic origin	Balfour (1876) (1877)	Scyllium Pristiurus	Germ cells are probably of mesodermal origin.
	Van Wijhe (1889)	Scyllium Pristiurus	Germ cells are derived from the segmental mesoderm (gonotome).
		PISCES	
Early segregation	Eigenmann (1891) (1896)	Micrometrus aggregatus	Germ cells can be traced back to the 5th or 6th cleavage.
	Dodds (1910)	Lophius piscatorius	Germ cells are first seen in the entoderm.
	Nussbaum (1880)	Trout	Germ cells are first seen in the region of the germ gland, but they are not derived from mesoderm.
	Reinhard (1924)	Scardinius	Germ cells arise from giant cells of the periblast.
	Hann (1927)	Cottus bairdii Girard	The primordial germ cells are formed from entodermal giant cells.
Somatic origin	MacLeod (1881)	Hippocampus Belone	Germ cells are derived from the germinal epithelium.
	Hoffmann (1886)	Salmon	Germ cells are derived from peritoneal cells.
	Böhi (1904)	Trout Salmon	Germ cells are derived from cœlomic epithelial cells.

	Author and year	Species	Remarks
		PISCES (continued)	
Somatic origin	Essenberg (1923)	Xiphophorus helleri	In the female, the germ cells are derived partly from the epithelium of the ovarian cavity and partly from cells of the cortex; in the male, they are derived from the epithelium of the tubules and from free peritoneal cells of the gonad.
	Foley (1927)	Umbra limi (male)	Spermatogonia are formed from cells in the stroma of the testis.
		AMPHIBIA	
Early segregation	Nussbaum (1880)	Rana fusca	Germ cells are first found in the mesoderm.
	Allen (1907)	Rana pipiens	Germ cells are first found in the entoderm.
	King (1907) (1908)	Bufo lentiginosus	Germ cells are first found in the entoderm.
	Witschi (1914)	Rana temporaria	Germ cells are first found in the entoderm.
	(1929)	Rana sylvatica	All definitive sex cells are early segregated from the entoderm.
	Burns (1925)	Ambystoma punctatum	All definitive germ cells are derived from early segregated cells.
	Cheng (1932)	Rana cantabrigensis	All germ cells are early segregated cells.
Somatic origin	Dustin (1907) (1907)	Rana fusca Bufo vulgaris	Germ cells are derived from the gonotome and from the peritoneum.
	Gatenby (1916)	Rana temporaria	Germ cells originate periodically from peritoneal cells in mature frogs.
	Obreshkove (1924)	Diemyctylus viridescens	There is strong evidence that peritoneal cells may transform into germ cells.
	Hargitt (1924)	Diemyctylus viridescens	The definitive germ cells in the male are derived from: a) the epithelial cells of the collecting ducts; b) the germinal epithelium.
	McCosh (1930)	Ambystoma maculatum	The majority of the germ cells are believed to be of somatic origin. The primordial germ cells may form a small portion of the propagative cells.

	Author and year	Species	Remarks
		REPTILES	
Early segregation	Allen (1906) (1907) (1911)	Chrysemys marginata	The early segregated germ cells are first found in the entoderm.
	Jarvis (1908)	Phrynosoma cornutum	The primordial germ cells are first seen in the entoderm.
	Risley (unp. mss.)	Sternotherus odoratus	Germ cells are early segregated cells.
Somatic origin	Dustin (1910)	Chrysemys marginata	Secondary germ cells come from peritoneal cells.
	Von Berenberg-Gossler (1914)	Lacerta agilis	Germ cells are derived from mesoderm. The so-called primordial germ cells are probably not germ cells.
	Simkins (1925)	Trionyx	There is no evidence for an extraembryonic origin of germ cells.
		BIRDS	
Early segregation	Nussbaum (1901)	Chick	Germ cells are first seen in the splanchnopleure.
	Rubaschkin (1907)	Chick, Duck	Germ cells are first seen in the splanchnopleure. They can be recognized by their Golgi bodies.
	Swift (1914)	Chick	Germ cells are first seen in the germ wall entoderm along the margin of the proamnion.
	Goldsmith (1928)	Chick	Germ cells are of extraembryonic origin and these cells give rise to the definitive sex cells.
	Dantschakoff (1931)	Chick	The primordial germ cells are identical with the entoderm wandering cells. They are necessary for gonad formation.
	Matsumoto (1932)	Chick	Primordial germ cells are first found in the posterior portion of the primitive streak and primitive plate in embryos of ten hours incubation.
Somatic origin	Waldeyer (1870)	Chick	Ova are derived from the germinal epithelium; spermatogonia, from the epithelium of the Wolffian duct.

	Author and year	Species	Remarks
		BIRDS (continued)	
Somatic origin	Von Berenberg-Gossler (1912)	Chick	The so-called primordial germ cells first found in the splanchnopleure are probably not germ cells.
	Firket (1914) (1920)	Chick	Most of the definitive germ cells come from the germinal epithelium. Some may come from the primordial germ cells.
		MAMMALS	
Early segregation	Rubaschkin (1908) (1909) (1912)	Cat, rabbit, mole, Guinea pig, porpoise	Primordial germ cells are first seen in the entoderm. These give rise to the definitive sex cells.
	Vannemann (1917)	Armadillo	The early germ cells are seen between the ectoderm and entoderm when the primary buds of ectodermic vesicles are in process of formation.
	Cowperthwaite (1925)	Rat	No cyclical proliferation of propagative cells from the germinal epithelium in adults was observed.
Somatic origin	Allen (1904)	Pig, rabbit	All functional germ cells come from peritoneal cells.
	Winiwarter & Sainmont (1909)	Cat	The definitive germ cells are derived from the germinal epithelium.
	Hargitt (1925)	Rat	The germ cells come from the peritoneum of the genital ridge.
	Butcher (1927)	Mus norvegicus albinus	All primordial germ cells degenerate. Definitive sex cells come from the germinal epithelium.

brates a real "Keimbahn" as has been demonstrated to exist for invertebrates. In drawing up the preceding tables, therefore, this question was kept uppermost in mind, and the diverse theories that support the origin of any germ cells from other sources except from the primordial germ cells are necessarily grouped together. In this classification, therefore, groups I, II, and III of Heys are combined and contrasted with her group IV. Any necessary qualifications are dealt with under appropriate "remarks". In drawing

up these tables, no special attempt has been made to include all the papers that have appeared in the literature of this vast field, but the examples have been selected mainly to show that early segregation and somatic origin of germ cells have been claimed for representatives of all classes of vertebrates, and that contradictory claims have even been made for the origin of the germ cells in the same species.

REVIEW OF THE LITERATURE ON THE LATER HISTORY OF THE PRIMORDIAL GERM CELLS AND DISCUSSION.

As far as many authors are concerned, the question of the origin of the definitive sex cells in vertebrates is quite distinct from that of the origin of the primordial germ cells. Many investigators, while willing to admit that the germ cells appear early in the ontogeny of the individual in all classes of vertebrates, do not look upon these early segregated cells as the progenitors of the sex cells in the adult. The question itself seems quite paradoxical; for if the primordial germ cells do not give rise to the definitive sex cells, why should they be looked upon as germ cells at all? The difficulty of the dilemma was recognized by several investigators and the easiest way out seemed to be the denial of the sex nature of these cells.

Von Berenberg-Gossler (1914), from his work on Lacerta, concludes that the primordial germ cells are not germ cells at all, but represent a late transformation of entodermal cells into mesodermal cells. After this transformation has once taken place, they, like any other mesodermal cells, may become stem cells which give rise to male or female sex cells. He says "dass die Auswanderung von Zellen aus dem Entoderm nichts anderes bedeutet als eine späte noch längere Zeit hinziehende Mesodermbildung aus dem Entoderm." In the duck, he considers the sex nature of these cells very doubtful, but he is not prepared to say whether the entodermal wandering cells of the duck are homologous with those of Lacerta or not. He concludes, however, "dass man von einer Keimbahn bei den Sauropsiden nicht mehr reden kann."

Among those who share the opinion of von Berenberg-Gossler, or hold similar views, may be mentioned, Simkins (1923), Hargitt (1925), and Stieve (1927). Stieve, especially is very emphatic in his denial of the sex nature of these cells when he says: "Die primordialen Geschlechtszellen einiger Forscher sind, wie schon v. Berenberg-Gossler gezeigt hat, entodermale Wanderzellen, die an den verschiedensten Stellen des Körpers, besonders im Bereiche des kaudalen Darmschnittes aus dem Entoderm entstehen, in das Mesenchym abgegeben werden und dort verschwinden. Mit der Keimzellbildung haben sie nicht das geringste zu tun." Since the authors

just considered deny the sex nature of the primordial germ cells, they quite naturally hold to a somatic origin for the true sex cells.

There is, however, a large class of workers who admit that the primordial germ cells are true sex elements, some of which may give rise to ova and spermatozoa, but believe that these can also come from somatic sources. Those who subscribe to this view constitute the large class whose opinions fall in the "middle ground" of McCosh (1930). It embraces a variety of opinions and combinations of opinions almost as numerous as the authors themselves. Chief among those who would not have any of the primordial germ cells arrive at sexual maturity, is Firket (1914, 1920 b), who makes a sharp distinction between the primary gonocytes, which are extraembryonic in origin and later migrate to the gonads, and the secondary gonocytes which arise from the germinal epithelium and give rise to the definitive sex cells. He finds that in the chick, at a certain stage, it is impossible to distinguish one generation from the other, and that it is, therefore, impossible to say whether or not any of the first generation of cells ever develop into mature sex cells. In the testis of the white rat, however, he finds that the same two generations of cells occur, but before the secondary germ cells appear, the first generation has entirely disappeared. Firket does not deny the sex nature of the primordial germ cells, since he says that they pass through the stages of maturation, typical of true sex cells. From a comparison of his works on the chick and on the rat, he holds it very probable that none of the primordial germ cells in either species become definitive sex cells, and that they are of only phylogenetic value. He says: "The primary germ cells disappear in the ontogenesis of mammals earlier than they do in the ontogenesis of birds and can therefore be considered as being cells in a phylogenetic regression."

Firket's contention that the primordial germ cells degenerate and are replaced later by definitive germ cells of somatic origin receives considerable support from the results of other workers, particularly in the field of mammals. Butcher (1927) believes that in the white rat, *Mus norvegicus albinus*, the germ cells found in the ovary at birth all degenerate before sexual maturity and are, therefore, not the progenitors of the definitive ova. The definitive ova, according to his views, are formed from the germinal epithelium covering the ovary, and the process of ova formation extends from six to seven days after birth until fecundity is lost in old age. The same author also carried out investigations on the germ cells in the lake lamprey, *Petromyzon marinus unicolor* (1928, 1929). He is ready to admit that in this species some of the primordial germ cells become definitive sex cells, but contends that the finding of successive stages from the ordinary epithelial cell to the definitive germ cell offers evidence that germ cells are also formed from the peritoneal cells.

Taking the vertebrate group as a whole, most of the workers on germ cells who take the "middle ground" of McCosh hold to a dual origin for the definitive sex cells. Opinions in this group are as interesting as they are numerous. Dustin (1907, 1910) makes the same distinction between primary and secondary gonocytes in amphibians and reptiles as Firket does for birds and mammals. In his work on amphibians (1907), he says: "Chez les amphibiens, il existe deux lignées de gonocytes: une première provenant des ébauches mésoblastiques primaires, une seconde provenant de l'épithelium cœlomique qui a proliféré." He admits, however, that in both of these classes of vertebrates, some of the first generation of gonocytes give rise to definitive sex cells. Gutherz (1919) has found a dual origin of germ cells in the ovary of the cat. He maintains that some of the oögonia found in the ovary three weeks after birth can be traced back directly to the primordial germ cells; but on the other hand, transformation of epithelial cells into germ cells can be followed step by step. Wolf (1931) reaches a quite novel conclusion as to the origin of the definitive sex cells in the teleost, *Platypœcilus maculatus.* He says that there is some evidence of a transformation of somatic cells into germ cells in the immature female, but that this transformation is not extensive. In the male, he holds the primordial germ cells to be the sole source of the definitive sex elements.

Those who hold to a somatic origin of sex cells all believe, either implicitly or explicitly, that there must exist transitional stages between the sex cells and the somatic cells from which they are said to be derived. Gatenby (1924), Gutherz (1919), Butcher (1927, 1929) and others claim to have observed these stages, but it has been pointed out by Beard (1904) and Dantschakoff (1931 a) that the evidence in support of this contention is not conclusive. Beard says that "they have never been figured, because they have no existence in fact." From his own work on the germ cells of *Raja batis,* he is very emphatic about the non-existence of these transition cells. He says: "After prolonged and careful investigation of the origin and early history of the primary germ cells in Raja, conducted after the best and most exact methods, it must be emphatically stated that in this animal there is no particle of evidence to be found pointing to an origin of a single germ cell from any cell or cells of the embryo." We may not feel justified in applying the results discovered in one form without reservation to other forms, especially to those belonging to different vertebrate classes, but we can certainly agree with Dantschakoff when she says: "Eine blosse Behauptung, dass ungemein viele Übergangsstadien anwesend sind (Stieve) genügt nicht. ... *Der* ist des Beweises schuldig, der eine Behauptung vorbringt."

A variety of opinions concerning the origin of the sex elements, have also been advanced by workers on adult material. In those vertebrates that

undergo seasonal changes in the gonads, many have held that during the period of sexual inactivity, all germ cells degenerate, and that the sex cells arise anew from somatic sources at the onset of renewed reproductive activity. OBRESHKOVE (1924) holds that in *Diemyctylus viridescens* there is strong evidence that peritoneal cells may transform into germ cells and initiate the development of new testicular lobes. BURNS (1925), on the other hand, noticed that in *Amblystoma punctatum* nearly all the germ cells disappear during sexual inactivity, but he adds that "in preparation for the next breeding season, the germ cells are regenerated from residual spermatogonia which survive the process of degeneration."

My own observations on the germ cells of *Passer domesticus* have lent no support whatever to the theory of transformation of somatic cells into germ cells. The germ cells which were traced from their place of segregation to the gonads, have been studied at all stages of incubation in both sexes to the time of hatching. Not a single instance of a "transitional cell" was discovered. The increase in numbers is due solely to multiplication of these cells by mitosis. KUMMERLÖWE's (1930 b) work on the post-embryonic development of the female gonads of the English sparrow makes no mention of ova arising from the somatic cells, but seems to take it for granted that the ova found in the adult are direct descendents of the primordial germ cells. The history of the germ cells in *Passer domesticus,* from their first appearance to adult ova and spermatogonia, seems to be in close harmony with the results of GOLDSMITH's (1928) work on the germ cells of the chick.

To test some of the current theories on the ultimate fate of the primordial germ cells as well as on their origin, recourse has been had in recent years to the experimental method. The experiments of REAGAN, WILLIER, and DANTSCHAKOFF on early chick embryos, in which the entoderm wandering cells are identified with the primordial germ cells, have already been referred to. Many of the early investigators on the history of the germ cells in vertebrates carried their researches only to the stage when the primordial germ cells had arrived at their destination in the anlagen of the gonads, and thus missed the real point at issue. GUTHERZ (1919) clearly saw that any really important work on the "Keimbahn" in vertebrates must also take into consideration the ultimate fate of the early germ cells, from whatever source they may have been derived. He states the problem as follows: "So ist für die Wirbeltiere und für den Menschen das frühzeitige Auftreten isolierter Geschlechtszellen im Embryo fast allgemein anerkannt, und der Streit dreht sich im wesentlichen darum, ob diese Zellen nicht später wieder schwinden und in der Geschlechtsdrüse durch Neubildung aus dem Coelomepithel ersetzt werden." To help settle this problem definitely in birds, the experimental work of DANTSCHAKOFF (1931 c) on the germ cells of the chick is of the greatest importance. She found that transplants of early gonad an-

lagen of two day embryos, that is before the arrival of the primordial germ cells in this region, on to the chorio-allantois of normal embryos, not only contained no germ cells at the end of fifteen days of incubation, but that the gonads also failed to develop. She found, moreover, that if the early gonads are destroyed, primordial germ cells will collect in great numbers in the splanchnopleure anterior to the destroyed gonads; if this tissue with its primordial germ cells is transplanted to the chorio-allantois of normal embryos, a normal gonad develops *with germ cells in its tubules.* From her experiments, two obvious conclusions can be drawn: a) The primordial germ cells are necessary to stimulate the germinal epithelium to gonad formation; b) The germinal epithelium (of WALDEYER) is of itself unable to give rise to sex cells. Her work, therefore, gives strong support to the theory of early segregation of germ cells, and tends to show that in the chick, the definitive sex cells are in a direct line with the primordial germ cells of earlier stages and that none are derived from the germinal epithelium.

My own observations on the germ cells of *Passer domesticus* revealed no extensive degeneration of germ cells. All germ cells found in the ovary or testes at the time of hatching can be traced back in an unbroken line to the primordial germ cells found in the germinal area anterior and antero-lateral to the embryo in the one to eight somite stages.

SUMMARY OF OBSERVATIONS AND CONCLUSIONS.

1. The primordial germ cells of *Passer domesticus* are of extraembryonic origin and are first seen in an embryo of one or two somites in a crescent-shaped area at the outer margin of the proamnion. They are found in the entoderm and in the space between the entoderm and the ectoderm.

2. They remain in this position up to the seven or eight somite stage during which time they take on more definite germ cell characteristics.

3. After the arrival of the mesoderm, the germ cells enter the blood vessels of the vascular area, in part passively during the formation of the blood islands and in part actively by forcing their way through the endothelial lining of these vessels. In a ten somite embryo, all stages in the process of this migration can be observed.

4. The germ cells are carried through the circulation with the blood cells, and at about the twenty-five somite stage, make their appearance in the small vessels of the splanchnopleure where they leave these vessels and begin their migration toward the site of the future gonad, where some of the germ cells take their place among the cells of the so-called germinal epithelium.

5. The lodging of the germ cells in the vessels of the splanchnopleure is attributed solely to mechanical factors, and is dependent upon the size and shape of the vessels in this region.

6. The progress of migration of the germ cells from the vessels of the splanchnopleure continues to about the thirty-six somite stage, when practically all of the germ cells have left the blood vessels. A small number of germ cells become lodged in small vessels of distant regions and probably never reach the gonads.

7. There is a shifting of the germinal epithelium with its contained germ cells from the splanchnopleure, through the cœlomic angle, to the somatopleure during the stages from three to four days incubation.

8. During the formation of the gonads, and during sex differentiation, germ cells are found in all parts of the gonads. The first mitoses of germ cells were observed in a four and one half day embryo.

9. During sex differentiation, there is no distinct formation of tubules as has been described for the chick. The first reliable criterion of sex distinction is the relative size of the right and the left gonad in the female.

10. A rudiment of the right gonad of the female with germ cells persists at all stages of incubation. It retains its undifferentiated structure throughout this period.

11. Histologically, sex cannot be distinguished before the eighth day of incubation, when a cortex begins to form beneath the epithelium of the left ovary.

12. The primordial germ cells are not replaced by a second generation of germ cells, but they give rise directly to the definitive sex elements. Their number is increased only by mitosis and no germ cells are derived from somatic sources.

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PLATES.

ABBREVIATIONS.

adr., adrenal gland.
b.c., blood cell.
b.v., blood vessel.
c.a., cœlomic angle.
cor., cortex.
d.a., dorsal aorta.
ect., ectoderm.
end., endothelium.
ent., entoderm.
ep., epithelium.
exc., exocœlc.
g.a., germinal area.
G.C., germ cell.
g.e., germinal epithelium.
g.ent., gut entoderm.
l.g., left gonad.
l.o., left ovary.
l.t., left testis.
med., medulla.
meta., metanephros.
mes., mesoderm.
msn., mesentery.
msnp., mesonephros.
ncd., notocord.
p.c.v., posterior cardinal vein.
pro., proamnion.
p.v.c., posterior vena cava.
r.g., right gonad.
r.o., right ovary.
r.t., right testis.
s.c.v., sub-cardinal vein.
sm., somite.
sm.ms., somatic mesoderm.
sp.ms., splanchnic mesoderm.
v.art., vitelline artery.
v.v., vitelline vein.
W.d., Wolffian duct.

All outlines were made with a camera lucida. All drawings, unless otherwise stated, are of transverse sections.

EXPLANATION OF FIGURES.

PLATE I.

1. Generalized diagram of an early sparrow blastoderm. The black area shows the region where the primordial germ cells are first found.

2. Section through the germinal area of a two-somite embryo, showing the germ cells at their earliest appearance. The plane of the section passes through *a*—*a* of figure 1. (× 690.)

3. Section through the germinal area of a seven-somite embryo, slightly anterior to the plane of the section shown in figure 2. (× 690.)

4. Transverse section of one half of the blastoderm of a ten-somite embryo passing through the head region. The figure is given for orientation of figures 5, 6, and 7, which are taken from the mesoderm at the outer margin of the exocœle. (× 70.)

5. Part of the section shown in figure 4, showing a germ cell at the outer margin of a blood vessel in the splanchnopleure. (× 690.)

6. Section of the same region as figure 5, showing the breaking down of the endothelial lining of a blood vessel by an entering germ cell. (× 690.)

7. Section showing a germ cell among embryonic blood cells in a blood vessel of the splanchnopleure of a ten-somite embryo. (× 690.)

PLATE II.

8. Portion of a section through a twenty-two somite embryo showing a germ cell together with two blood cells in a blood vessel of the splanchnopleure. (× 690.)

9. Section showing a germ cell in a small artery from the dorsal aorta in the splanchnopleure of a twenty-five somite embryo. (× 690.)

10. Section showing a germ cell stopped in its course in a small vessel of the splanchnopleure of a twenty-five somite embryo. Note the broken endothelium of the vessel. (× 690.)

11. Part of a section of a twenty-five somite embryo showing a germ cell in the splanchnic mesoderm just outside of a small vessel from the dorsal aorta. (× 690.)

12. Section showing a germ cell in the splanchnic mesoderm of a twenty-five somite embryo. (× 690.)

13. Section showing a germ cell in an omphalomesenteric artery on its way back to the vascular area of the blastoderm of a twenty-five somite embryo. (× 690.)

14. Section of the splanchnopleure of a thirty-two somite embryo showing a germ cell almost completely filling a small blood vessel; also two other germ cells in the mesenchyme. (× 690.)

15. Section of a part of the splanchnopleure of a twenty-nine somite embryo showing a large blood cell closely surrounded by mesenchyme cells. (× 690.)

16. Section showing a group of six germ cells in the splanchnopleure of a thirty-two somite embryo. Note the amœboid shape of the germ cells. (× 690.)

PLATE III.

17. Section through the posterior region of a four-day embryo showing the position of the germinal epithelium and the unequal distribution of the germ cells to the right and left sides of the embryo. The germ cells are represented in black. (× 65.)

18. Section of a thirty-three somite embryo showing two germ cells entering the epithelium of the somatopleure. Note the disarranged epithelial cells in contact with the germ cells. (× 690.)

19. Section showing a germ cell in a position among the epithelial cells of the somatopleure of a thirty-three somite embryo. (× 690.)

20. Section through the closed gut region of a thirty-four somite embryo showing a germ cell among blood cells in a small vessel of the mesoderm of the closed hind gut. (× 690.)

21. Section through a thirty-four somite embryo showing a germ cell migrating out of a small blood vessel in the mesoderm of the mesentery.

22. Section through a thirty-four somite embryo in the anterior region of the germinal epithelium. The germinal epithelium is represented in black. (× 80.)

23. Section through the same embryo as figured in 22 160 micra posterior to the section shown in figure 22. The germinal epithelium is represented in black. (× 80.)

PLATE IV.

24. Frontal section through the gonads and Wolffian bodies of a six-day embryo showing the difference in size of the right and the left gonad and the attachment of the gonads to the Wolffian bodies. (× 21.)

25. Section through the gonads of an eight-day female embryo showing the beginning of the cortex in the left gonad. (× 50.)

26. Section of a germ cell in mitosis from the gonad of a four and one half day embryo. (× 690.)

27. Section of an eleven-day male embryo through the anterior region of the gonads showing the disposition of the organs of the urogenital system. (× 32.)

28. Section of an eleven-day female embryo through the mid-region of the gonads. Note the difference in size between the right and the left gonad. (× 32.)

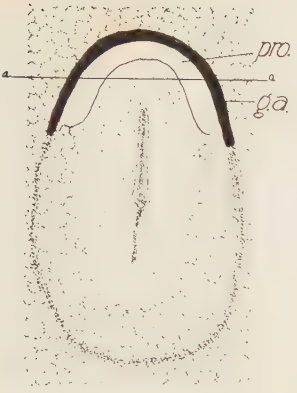
PLATE V.

29. Section of a portion of the left gonad of a six-day embryo. At this stage the right and the left gonads are identical in histological structure. (× 635.)

30. Section showing the histological structure of a portion of the left ovary of an eleven-day embryo. Three germ cells are shown. (× 635.)

31. Section of a portion of the right gonad of the same embryo as shown in figure 30. Note the thicker epithelium and deeper position of the germ cells. (× 635.)

32. Section showing the histological structure of a portion of the testis of an eleven-day male embryo. Cell outlines of the germ cells are not distinct but three germ cell nuclei stand out prominently among the cells of the forming tubules. (× 635.)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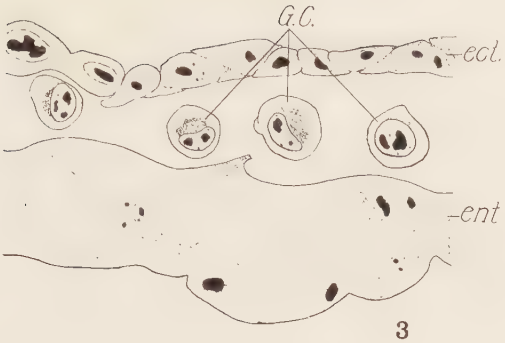
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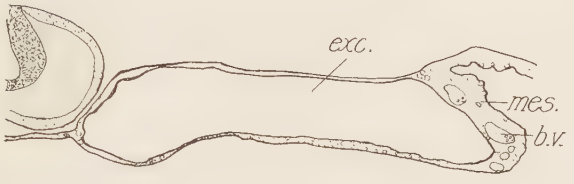
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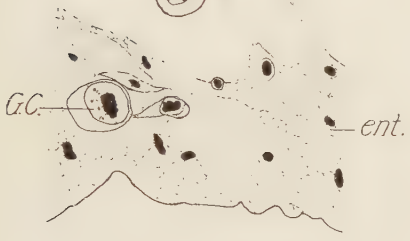
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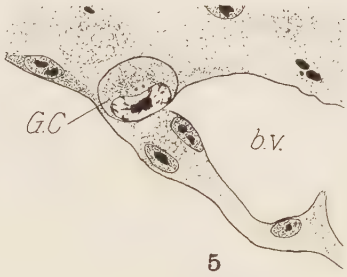
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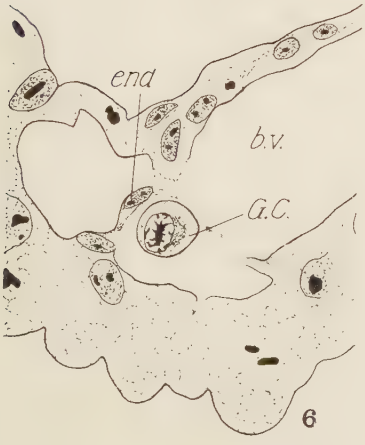
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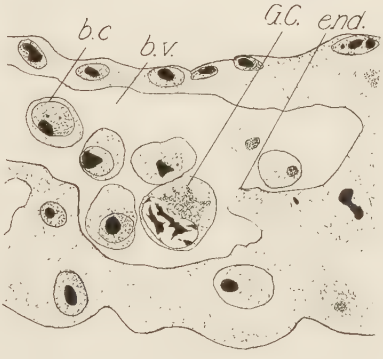
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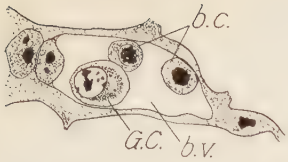

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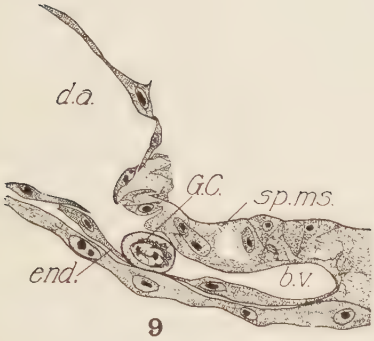

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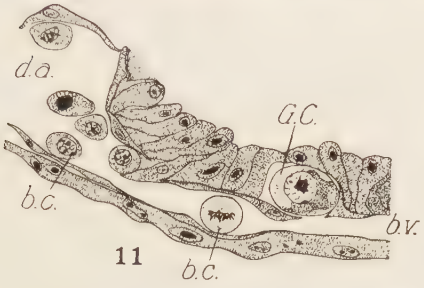

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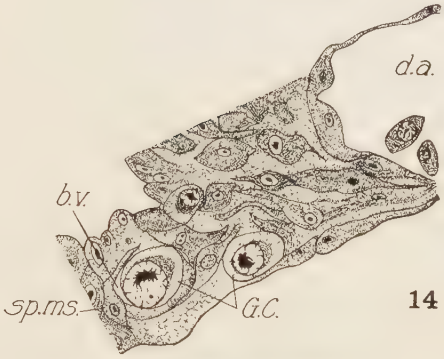

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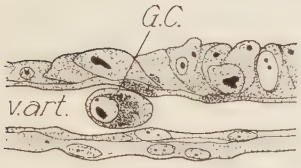

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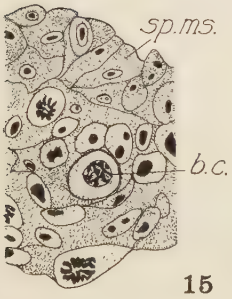

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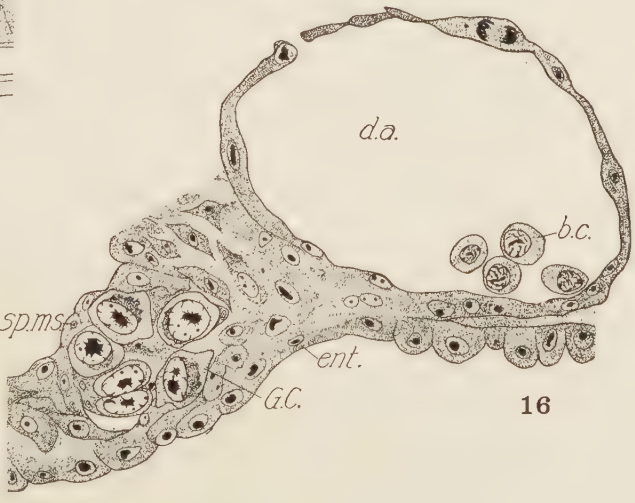

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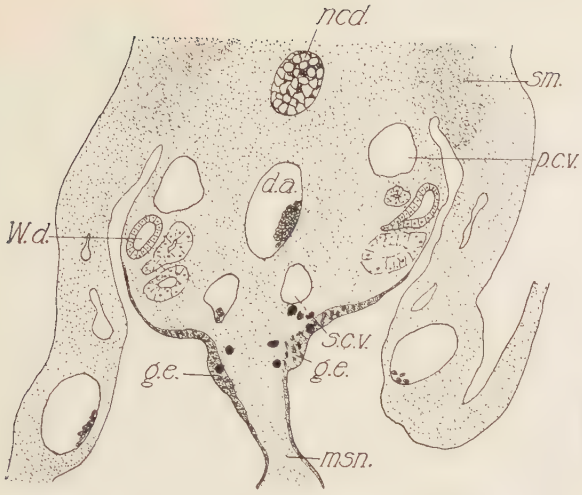
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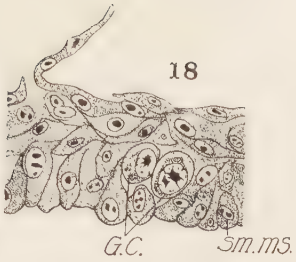
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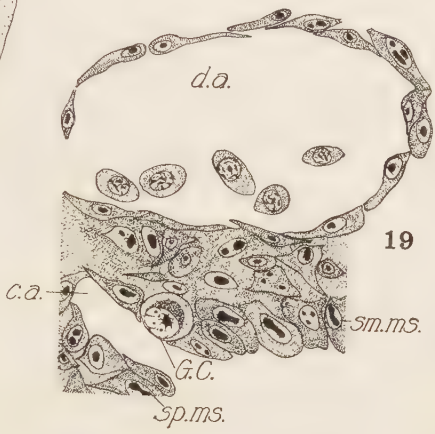
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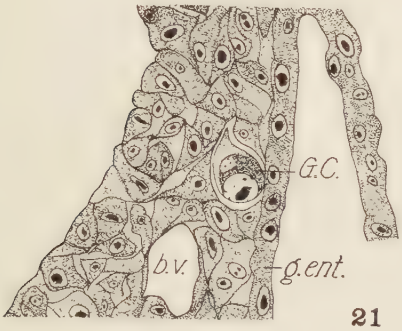
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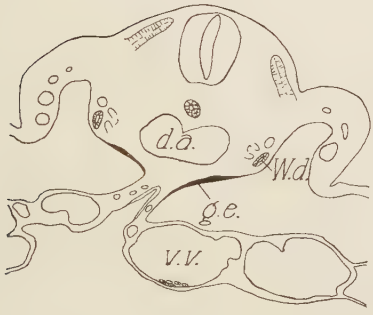
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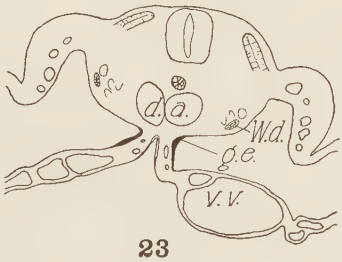
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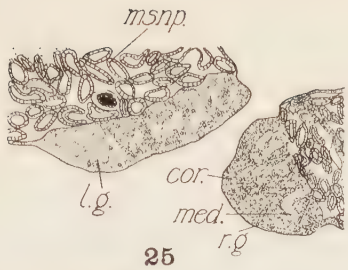
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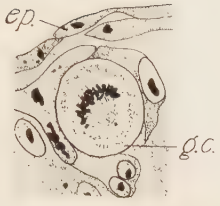
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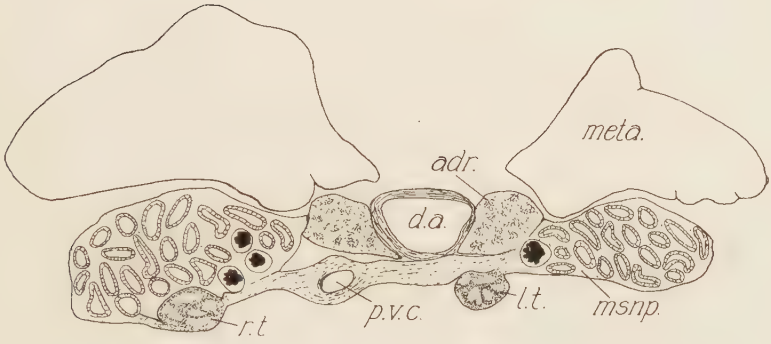
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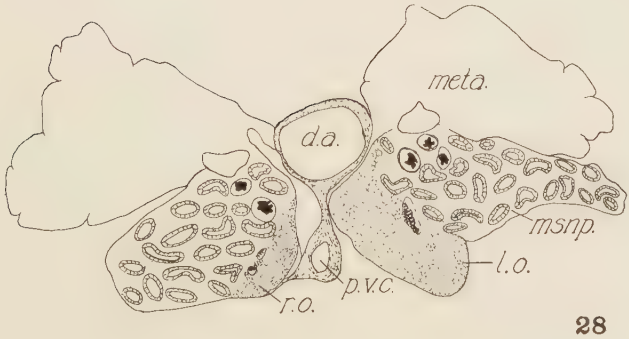
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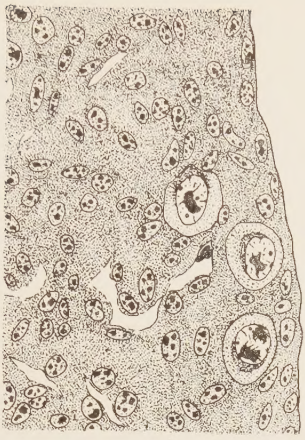

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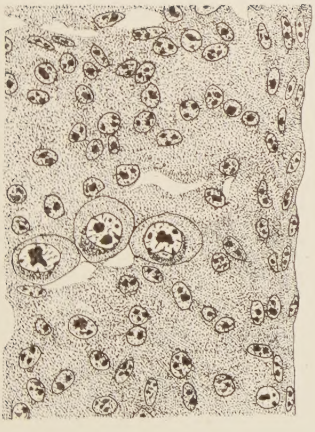

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